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From Fruit Flies to Barnacles, Histamine Is the Neurotransmitter of Arthropod Photoreceptors

Minireview

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Photoreceptor synapses of both vertebrate and invertebrate eyes are unconventional. These synapses release transmitter continuously yet never fatigue, and they transmit tiny presynaptic signals faithfully, often over a wide intensity range. Insight into these processes may now come with the identification of histamine as the neurotransmitter used by the photoreceptors of arthropod eyes. This minireview calls attention to peculiarities of arthropod photoreceptor synapses, reviews the evidence that histamine is the transmitter, and points out what we know of how histaminergic transmission is adapted to photoreceptor function.

The Histaminergic Photoreceptor Synapse, like the Glutamatergic Synapse of Retinal ON Bipolar Cells, Is an Unconventional Inhibitory Synapse

Photoreceptors throughout the animal kingdom function with graded potential changes rather than action potentials, release transmitter continuously whether in the dark or in the light, and increase transmitter release when depolarized, like conventional neurons. However, vertebrate and arthropod photoreceptors function precisely opposite from one another in their responses to light. Vertebrate photoreceptors rest at a depolarized membrane potential in the dark and hyperpolarize in response to light. This hyperpolarization reduces the release of the transmitter glutamate, causing reduced excitation of OFF bipolar cells (where glutamate is excitatory) and disinhibition of ON bipolar cells (where glutamate is inhibitory). Arthropod photoreceptors are depolarized by light and hyperpolarize in response to dimming or dark (Figure 1). The predominant cell type postsynaptic to arthropod photoreceptors resembles the vertebrate ON bipolar cell in being inhibited by the transmitter released when the photoreceptor is depolarized (histamine) and disinhibited in response to photoreceptor hyperpolarizations (Figure 1). Because the postsynaptic depolarizing signal at these inhibitory synapses is generated by the *reduction* of the transmitter concentration in the cleft, what assumes importance in its generation is not transmitter release from the photoreceptors but the expeditious *interruption* of transmitter release; not the surge of transmitter into the cleft and its binding to postsynaptic receptors but the quick clearance of transmitter by diffusion or uptake and its *dissociation* from the postsynaptic receptor; not the opening of postsynaptic channels but how rapidly they close.

Like its counterpart in the vertebrate retina, the arthropod synapse is "high gain" in that miniscule changes in presynaptic potential can lead to considerably larger changes in postsynaptic potential. In addition, barnacle and insect photoreceptor synapses have been shown to adapt, centering their operating range on the value of presynaptic voltage set by the background light intensity. Even when the photoreceptor is maintained in a depolarized state in bright lights, these hardy synapses do not fatigue (Hayashi et al., 1985). This intriguing synaptic property focuses interest not only on the mechanism of adaptation itself, which is likely to involve modulation of the release, reception, or recycling of transmitter, but also on the trick of maintaining an apparently infinite transmitter supply.

The Evidence for Histamine as the Photoreceptor Transmitter Is Increasingly Tight

Long known as a busy molecule in the periphery, histamine was fingered in the 1970s as a neurotransmitter candidate in the mammalian CNS and in *Aplysia* neurons (reviewed by Schwartz, 1991). The observation that histamine hyperpolarized postsynaptic cells in the fly (Hardie, 1987, 1988) triggered an avalanche of evidence in favor of histamine as the major photoreceptor transmitter in arthropods. Unlike glutamate, the long-elusive transmitter of vertebrate rods and cones, histamine is a highly specialized molecule, rare in neurons. This uniqueness has permitted researchers to satisfy quickly almost all criteria for its establishment as a neurotransmitter.

The immense variety within the arthropods presents a banquet of eyes and approaches from which to choose in probing the details of histamine's function. Photoreceptors from all of the arthropod classes—the crustacea, the insects, and the arachnids (spiders and horseshoe crabs)—immunolabel for histamine and are presumed histaminergic. The laminated compound eye of flies provides dozens of postsynaptic cells that may be dissociated and patch clamped to study histamine's postsynaptic effects (Hardie, 1989; Skingsley et al., 1995). The huge photoreceptors of barnacles contribute large presynaptic terminals for studying how histamine is synthesized, released, and recycled (Stuart and Callaway, 1991; Stuart et al., 1996; Morgan et al., 1999). *Drosophila* visual mutants permit a genetic approach (Burg et al., 1993; Melzig et al., 1998). In locust, barnacle, and certain fly species, both presynaptic and postsynaptic cells are accessible for intracellular recording to explore how histamine mediates signal transfer at this type of synapse.

Photoreceptors Immunolabel for Histamine in a Wide Variety of Species

A well-characterized polyclonal antiserum against a histamine–carbodiimide–protein conjugate (Panula et al., 1988), used with great success in the mammalian CNS (reviewed by Schwartz et al., 1991), labels photoreceptors in both simple and compound eyes of cockroaches, spiders, bees, crickets, various flies, *Limulus* (where both retinular cells and eccentric cells label), and barnacles. Biochemical measurements from a number of these eyes reveal relatively high levels of endogenous histamine (about 2 nmol/mg protein).

The subcellular site of histamine storage has not been determined and may prove to be an unusual feature of histaminergic synapses. 30 nm clear vesicles are found at the release sites in the photoreceptor terminals of various insects and barnacles (with dense-core vesicles

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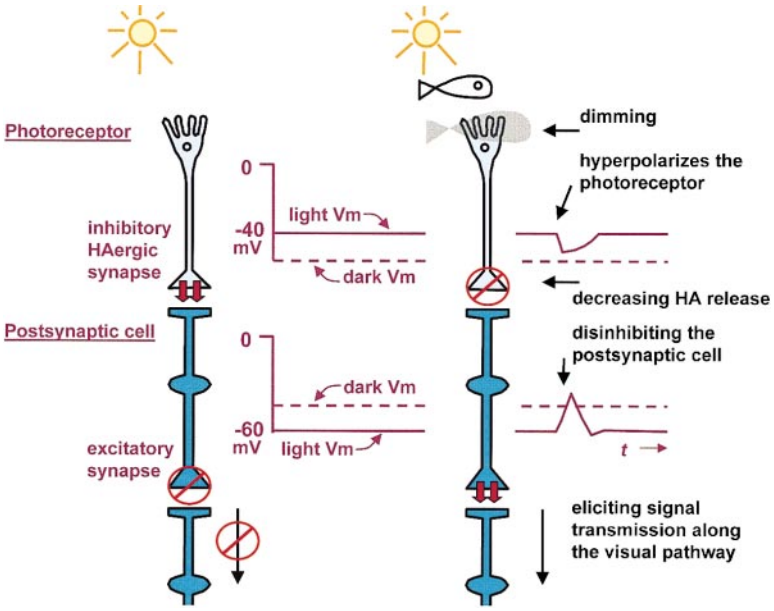

Figure 1. Disinhibition at the Histaminergic Synapse of an Arthropod Photoreceptor

This highly simplified scheme outlines the basic features of the first visual synapse for the barnacle, where shadows ultimately cause withdrawal of the animal into its shell. It ignores details of adaptation in the voltage of both the photoreceptor and the postsynaptic cell in order to focus on disinhibition.

behind) and also in *Aplysia* histaminergic (nonphotoreceptor) neurons. But, when the *Aplysia* neurons were injected with [^{3}H]histamine and analyzed by autoradiography, label accumulated over 100 nm dense-core vesicles (Schwartz et al., 1986). Do the dense-core vesicles store a reserve supply of histamine while the clear ones house the readily releasable pool? Questions such as this are unlikely to be answered until the development of methods that permit histamine's ultrastructural localization, such as an antiserum that will work at the EM level.

Histidine Decarboxylase Normally Synthesizes Histamine but Is Defective in Blind **Drosophila** *Mutants*

Histamine is generally synthesized in a one-step reaction from histidine by histidine decarboxylase (HDC). Photoreceptors injected with labeled precursor synthesized labeled histamine (Morgan et al., 1999). Despite the continuous release of transmitter from photoreceptors, the rate of uptake of precursor at these synapses is not greater than for nonhistaminergic neurons, and histamine synthesis proceeds at the casual pace of aminergic transmitters in general (Morgan et al., 1999). Presumably, the transmitter supply is maintained in the short run by reuptake of released histamine rather than by synthesis.

For several decades, it has been known that a large number of *Drosophila* visual mutants have defects in their electroretinograms that suggest dysfunction of the synapse made by the photoreceptors. Identification of histamine as the transmitter now provides a set of candidate molecules—e.g., HDC, the histamine receptor, and possible histamine transporters—as possible targets in these mutations. Indeed, one group of these mutants is deficient in histamine synthesis due to a defective HDC gene (*hdc*) (Burg et al., 1993); expression of the cloned *hdc* cDNA rescues the mutant phenotype in transgenic flies. Flies homozygous for the allele hdc^{JK910}, apparently a null mutation, show no histamine immunoreactivity in the photoreceptors and also are blind (Melzig et al., 1998). Another group is defective in the subcellular distribution and K^+-induced release of histamine (Lee et al., 1997, Soc. Neurosci., abstract). This genetic approach contributes strong evidence to the argument that histamine is the photoreceptors' transmitter.

Histamine Directly Gates a Postsynaptic Cl^- Channel

The receptor for histamine in arthropods is related by its pharmacology to the mammalian H2-type, G protein–coupled receptor (Hardie, 1987, 1988) but is a histamine-gated Cl^- channel. Patch clamp recordings from isolated postsynaptic cells of several fly species (Hardie, 1989; Skingsley et al., 1995) gave single channel conductances of 40–60 pS and brief open times ($<$1 ms). Histamine binds to its receptor cooperatively ($n = 1.8–2.8$) and with low affinity ($K_D = 24–50\ \mu M$), the values within this range depending on the species: faster-flying insects tended to have "faster" and more sensitive histamine receptors, characterized by lower histamine affinity and higher cooperativity (Skingsley et al., 1995).

At the ON bipolar synapse of the vertebrate retina, a postsynaptic cGMP cascade amplifies the presynaptic signal. It is not yet known whether histamine similarly initiates a cyclic nucleotide cascade to help mediate disinhibition.

Histamine Is Taken up Avidly and Selectively into Photoreceptor Terminals

Barnacle photoreceptors show specific, Na^+- and depolarization-dependent uptake of [^{3}H]histamine into their axons and presynaptic terminals (Stuart et al., 1996). Uptake into *Limulus* photoreceptors has also been shown (Battelle et al., 1999). In both of these eyes, the accumulated [^{3}H]histamine is retained for many hours and only slowly metabolized. Although uptake of histamine into *Drosophila* photoreceptors has not been demonstrated directly, probably because of a permeability barrier (e.g., Sarthy, 1991), Melzig et al. (1998) found that feeding histamine to mutant flies lacking HDC (and therefore

endogenous histamine) restored their vision and the immunolabeling of their photoreceptors. This clever experiment shows that uptake into *Drosophila* photoreceptors must exist.

Histamine Release from Eyes Can Be Demonstrated

Ca^{2+}-dependent release of [^{3}H]histamine (loaded into photoreceptors by uptake) in response to depolarization with high [K^+] has been shown for *Drosophila*, barnacle, and *Limulus* eyes (Sarthy, 1991; Stuart and Callaway, 1991; Battelle et al., 1999). It remains a sticking point, however, that light-dependent release of histamine from photoreceptors has not so far been demonstrated. In the case of *Limulus*, a blocker of histamine uptake was required during the high [K^+] challenge to enable detection of released histamine. It may be that uptake is so powerful that it prevents the collection of [^{3}H]histamine released in response to depolarization of the photoreceptors with light.

Conclusion

Histamine's actions at the photoreceptor synapse are well adapted to the requirements of this synapse. Histamine quickly inhibits the postsynaptic cell by directly gating a Cl^- channel, an atypical action since in vertebrates histamine works primarily through G protein–linked cascades. At the photoreceptor synapse, histamine binds loosely to its postsynaptic receptor so that it can dissociate quickly, permitting the channel closure that underlies the postsynaptic disinhibitory response. The histamine-gated Cl^- channels have brief open times, enabling them to close quickly as transmitter dissociates. A specific uptake mechanism replenishes histamine stores in the photoreceptor in an activity-dependent fashion; whether uptake affects the postsynaptic signal during disinhibition is unknown but of considerable interest. Future cloning and characterization of this transporter and the histamine receptor molecules, perhaps from the bank of *Drosophila* visual mutants, should lead to progress in understanding how high gain signaling is preserved at this synapse over a wide range of light intensities.

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